# Genetic stock identification of Chinook salmon mixtures at Bonneville Dam

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by

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**Summary:**

The objective of this project was to identify stock composition and run timing of summer and fall Chinook salmon passing Bonneville Dam. Included in this study was an evaluation of the cut-off date that designates the start of the summer run of Chinook salmon. A total of 1163 samples collected at Bonneville Dam from June 1 to October 20, 2005 were genotyped with 13 microsatellite loci. A genetic baseline of 55 populations was utilized to complete genetic stock identification of unknown origin samples from Bonneville Dam. Of the 450 samples collected as summer run Chinook (June 16 to July 19), the majority (90.2%) were from the mid/upper Columbia River reporting unit (Hanford Reach/Methow River) with very minor contribution from Snake River (1.6%) and Klickitat River (1.4%) reporting units. Of the 515 fall run samples collected from September 9 to October 20, a much larger proportion of the mixture was composed of Snake River (21.7%), Klickitat River (3.7%), and Deschutes River (3.0%) reporting units relative to the summer collection. However, the fall mixture was still dominated in composition by the mid/upper Columbia River reporting unit (70.1%). This study demonstrated the utility of GSI methods for evaluating stock composition of Chinook salmon mixtures from Bonneville Dam and potential for managing fisheries with this additional information.

**Introduction**

Genetic Stock Identification (GSI) methods have proven to be effective in determining the proportion of stock origin in several mixed stock fisheries (Shaklee et al. 1999, Beacham et al. 2006).  This study includes sampling unknown Chinook salmon at Bonneville Dam for genetic analysis.  Samples were collected over the entire length of the run on a weekly basis.  Current assignment of Chinook salmon to spring, summer and fall by date of passage is to some extent arbitrary.  However, few studies have been able to determine the extent of overlap among these life history types.  GSI of each life history type will allow us to determine the stock composition of the different runs through Bonneville Dam with greater accuracy than current methods.  Population genetic methods and statistical assignment models taking advantage of the power of microsatellite techniques have advanced dramatically in recent years, and estimates of stock composition is now possible using Bayesian or Maximum Likelihood methods (Kalinowski 2003).

*Current Project Objectives*

The specific objectives of this project were:

A. Estimate the stock composition of mixtures of summer and fall run Chinook salmon collected at Bonneville Dam.

B. Evaluate the cut-off date that determines the start of the summer run of Chinook salmon above Bonneville Dam.

**Methods**

*Samples*

Tissue samples (n = 1163) of adult Chinook salmon were collected in the adult trap at Bonneville from June 1 to October 20, 2005, two days per week.  DNA was

extracted from tissue samples, and the polymerase chain reaction (PCR) was utilized to

amplify 13 microsatellite loci (Table 1). Electrophoresis of fragments was used to detect

fluorescently labeled PCR products.  Raw genotype data was converted to standardized

allele names designated by the Genetic Analysis of Pacific Salmonids (GAPS)

consortium (Moran et al. 2005; Seeb et al. *in press*).

**Table 1.**  Microsatellite loci standardized for Chinook salmon

| Locus | Primer Sequence (5'→ 3')<br>**F** > Forward, **R** > Reverse | Citation |
|---|---|---|
| *Ots201b* | F- CAGGGCGTGACAATTATGC<br>R- TGGACATCTGTGCGTTGC | unpublished |
| *Ots208b* | F- GGATGAACTGCAGCTTGTTATG<br>R- GGCAATCACATACTTCAACTTCC | Grieg et al. 2003 |
| *Ots211* | F - TAGGTTACTGCTTCCGTCAATG<br>R - GAGAGGTGGTAGGATTTGCAG | Grieg et al. 2003 |
| *Ots212* | F- TCTTTCCCTGTTCTCGCTTC<br>R- CCGATGAAGAGCAGAAGAGAC | Grieg et al. 2003 |
| *Ogo4* | F- GTCGTCACTGGCATCAGCTA<br>R- GAGTGGAGATGCAGCCAAAG | Olsen et al. 1998 |
| *Ogo2* | F- ACATCGCACACCATAAGCAT<br>R- GTTTCTTCGACTGTTTCCTCTGTGTTGAG | Olsen et al. 1998 |
| *Ots3M* | F- TGTCACTCACACTCTTTCAGGAG<br>R- GAGAGTGCTGTCCAAAGGTGA | Banks et al. 1999 |
| *Ots213* | F- CCCTACTCATGTCTCTATTTGGTG<br>R- AGCCAAGGCATTTCTAAGTGAC | Grieg et al. 2003 |
| *Omm1080* | F- GAGACTGACACGGGTATTGA<br>R- GTTATGTTGTCATGCCTAGGG | Rexroad et al. 2001 |
| *Ssa408UOS* | F- AATGGATTACGGGTACGTTAGACA<br>R- CTCTTGTGCAGGTTCTTCATCTGT | Cairney et al. 2000 |
| *Ots9* | F- ATCAGGGAAAGCTTTGGAGA<br>R- CCCTCTGTTCACAGCTAGCA | Banks et al. 1999 |
| *OtsG474* | F- TTAGCTTTGGACATTTTATCACAC<br>R- CCAGAGCAGGGACCAGAAC | Williamson et al. 2002 |
| *Oki100* | F- CCAGCACTCTCACTATTT<br>R- CCAGAGTAGTCATCTCTG | unpublished |

*Mixture Analysis*

A genetic baseline of 55 populations (Table 2) was utilized to estimate stock composition of mixtures collected at Bonneville Dam.  The baseline included 51 populations from the Columbia River Basin, and four populations outside of the Columbia Basin (two from California, two from Alaska).  Baseline mixture simulations were examined with the program GMA (Genetic Mixture Analysis; Kalinowski, 2003) to evaluate the power of the baseline to analyze mixture samples.  First, simulations of 55 mixtures, each with 100% composition of the 55 baseline populations, were completed independently.  In each simulation, 400 multilocus genotypes were drawn from the baseline (sampling with replacement assuming random mating and independent assortment of loci) and stock composition of the mixture was estimated from the average of 1000 replicates, given 100% as the parametric value.  The second type of simulation included a mixture of five populations in the baseline, each with 20% composition. Again, 400 multilocus genotypes were drawn from the baseline as described above and stock composition of the mixture was estimated from the average of 1000 replicates, this time with 20% as the parametric value.  Stock composition estimates of the Bonneville samples were also estimated with the software program GMA.

The likelihood of each individual belonging to either spring, summer, or fall run was determined from each multi-locus genotype in the software program STRUCTURE (Pritchard et al. 2000).  Spring run samples were genotyped with PCSRF funds (cost share source), but added to this analysis for reference.

**Table 2**. Sample size and unbiased heterozygosity (He) for Columbia River Basin Chinook salmon populations included in the microsatellite baseline. Typical adult run timing for each population is given in parentheses.

|    | Population | n | He |
|----|------------|-----|-------|
| 1  | Cowlitz Hat. (fall) | 136 | 0.871 |
| 2  | Lewis R. (fall) | 93 | 0.886 |
| 3  | Sandy R. (fall) | 114 | 0.892 |
| 4  | Cowlitz Hat. (spring) | 133 | 0.861 |
| 5  | Kalama Hat. (spring) | 140 | 0.865 |
| 6  | Lewis Hat. (spring) | 125 | 0.866 |
| 7  | McKenzie Hat. (spring) | 138 | 0.817 |
| 8  | N. Santiam Hat. (spring) | 138 | 0.820 |
| 9  | Spring Cr. Hat. (fall tule) | 123 | 0.829 |
| 10 | upDeschutes R. (summer) | 141 | 0.868 |
| 11 | lowDeschutes R. (fall) | 126 | 0.872 |
| 12 | Carson Hat. (spring) | 129 | 0.792 |
| 13 | Warm Springs Hat. (spring) | 131 | 0.725 |
| 14 | Klickitat R. (spring) | 44 | 0.864 |
| 15 | Klickitat R. (summer) | 33 | 0.868 |
| 16 | Klickitat R. (fall) | 52 | 0.877 |
| 17 | Shitike Cr. (spring) | 136 | 0.763 |
| 18 | John Day R. (spring) | 127 | 0.780 |
| 19 | Yakima Hat. (spring) | 294 | 0.816 |
| 20 | Wenatchee R. (spring) | 110 | 0.795 |
| 21 | Methow R. (spring) | 90 | 0.793 |
| 22 | Entiat R. (spring) | 136 | 0.782 |
| 23 | Hanford Reach (fall) | 210 | 0.875 |
| 24 | Priest Rapids Hat. (fall) | 130 | 0.869 |
| 25 | Wells Hat. (fall) | 138 | 0.858 |
| 26 | Methow R. (summer) | 126 | 0.859 |
| 27 | Tucannon R. (spring) | 161 | 0.792 |
| 28 | Imnaha (spring) | 137 | 0.783 |
| 29 | Minam R. (spring) | 138 | 0.790 |
| 30 | Lostine R. (spring) | 101 | 0.754 |
| 31 | Catherine Cr. (spring) | 124 | 0.775 |
| 32 | Lyons Ferry Hat. (fall) | 137 | 0.870 |
| 33 | Clearwater R. (fall) | 110 | 0.856 |
| 34 | Nez Perce Tribal Hat. (fall) | 134 | 0.866 |
| 35 | Lolo Cr. (spring) | 109 | 0.787 |
| 36 | Newsome Cr. (spring) | 109 | 0.765 |
| 37 | Dworshak Hat. (spring) | 92 | 0.793 |
| 38 | Red River (spring) | 86 | 0.795 |
| 39 | Powell Trap (spring) | 138 | 0.788 |
| 40 | S. Fork Clearwater R. (spring) | 187 | 0.785 |
| 41 | Rapid River Hat. (spring) | 141 | 0.762 |
| 42 | Big Creek a (spring) | 69 | 0.754 |
| 43 | Big Creek b (spring) | 69 | 0.760 |

| 44 | Johnson Cr. (spring) | 143 | 0.776 |
| 45 | Secesh R. (spring) | 137 | 0.773 |
| 46 | McCall Hat. (spring) | 105 | 0.779 |
| 47 | Sawtooth Hat. (spring) | 181 | 0.790 |
| 48 | W. Fork Yankee Fork (spring) | 59 | 0.759 |
| 49 | E. Fork Yankee Fork (spring) | 141 | 0.769 |
| 50 | Pahsimeroi Hat. (spring) | 105 | 0.780 |
| 51 | Marsh Cr. (spring) | 46 | 0.782 |
| 52 | Battle Cr. (spring) | 122 | 0.841 |
| 53 | Butte Cr. (spring) | 123 | 0.792 |
| 54 | Klukshu R. | 139 | 0.764 |
| 55 | Situk R. | 129 | 0.759 |

## Results

A total of 18 reporting groups were identified for mixed stock analysis (Table 3) which includes 16 from the Columbia River Basin, one from California, and one from Alaska.  Results from 100% simulations had a range of 94.5-99.9% correct assignment for each of the 55 baseline populations (Table 3).  Accuracy was slightly higher with a narrower error range when the results were defined by reporting groups (98.0-100.0%; Table 3).  A mixture simulation with 20% population composition was within 1.23% of population composition and 0.19% of reporting unit composition.

Table 3. List of reporting units for each of the 55 baseline populations and the results from 100% simulations by Reporting Unit (RepUnit) and by Population.

| Reporting Unit | Population | Actual | Composition by RepUnit | Composition by Population |
|---|---|---|---|---|
| LowCol | Cowlitz Hat. (fall) | 1.0 | 0.9992 | 0.9950 |
| LowCol | Lewis R. (fall) | 1.0 | 0.9992 | 0.9957 |
| LowCol | Sandy R. (fall) | 1.0 | 0.9987 | 0.9953 |
| LowCol | Cowlitz Hat. (spring) | 1.0 | 0.9990 | 0.9976 |
| Willamette | Kalama Hat. (spring) | 1.0 | 0.9960 | 0.9957 |
| LewisHsp | Lewis Hat. (spring) | 1.0 | 0.9954 | 0.9954 |
| Willamette | McKenzie Hat. (spring) | 1.0 | 0.9998 | 0.9949 |
| Willamette | N. Santiam Hat. (spring) | 1.0 | 0.9998 | 0.9937 |
| LowCol | Spring Cr. Hat. (fall tule) | 1.0 | 0.9999 | 0.9995 |
| DeschutesOT | upDeschutes R. (summer) | 1.0 | 0.9981 | 0.9945 |
| DeschutesOT | lowDeschutes R. (fall) | 1.0 | 0.9910 | 0.9881 |
| upColST | Carson Hat. (spring) | 1.0 | 0.9935 | 0.9906 |
| midColST | Warm Springs Hat. (spring) | 1.0 | 0.9993 | 0.9992 |
| midColST | Klickitat R. (spring) | 1.0 | 0.9978 | 0.9978 |
| MidupColOT | Klickitat R. (summer) | 1.0 | 0.9983 | 0.9936 |
| MidupColOT | Klickitat R. (fall) | 1.0 | 0.9979 | 0.9909 |
| midColST | Shitike Cr. (spring) | 1.0 | 0.9983 | 0.9981 |
| midColST | John Day R. (spring) | 1.0 | 0.9902 | 0.9900 |
| YakimaST | Yakima Hat. (spring) | 1.0 | 0.9965 | 0.9965 |
| upColST | Wenatchee R. (spring) | 1.0 | 0.9961 | 0.9938 |
| upColST | Methow R. (spring) | 1.0 | 0.9929 | 0.9886 |
| upColST | Entiat R. (spring) | 1.0 | 0.9979 | 0.9972 |
| MidupColOT | Hanford Reach (fall) | 1.0 | 0.9808 | 0.9446 |
| MidupColOT | Priest Rapids Hat. (fall) | 1.0 | 0.9931 | 0.9632 |
| MidupColOT | Wells Hat. (fall) | 1.0 | 0.9966 | 0.9693 |
| MidupColOT | Methow R. (summer) | 1.0 | 0.9976 | 0.9760 |
| TucST | Tucannon R. (spring) | 1.0 | 0.9986 | 0.9986 |
| SFSalST | Imnaha (spring) | 1.0 | 0.9934 | 0.9927 |
| RapCWST | Minam R. (spring) | 1.0 | 0.9937 | 0.9808 |
| LostST | Lostine R. (spring) | 1.0 | 0.9992 | 0.9992 |
| RapCWST | Catherine Cr. (spring) | 1.0 | 0.9964 | 0.9870 |
| SnakeOT | Lyons Ferry Hat. (fall) | 1.0 | 0.9873 | 0.9566 |
| SnakeOT | Clearwater R. (fall) | 1.0 | 0.9898 | 0.9681 |
| SnakeOT | Nez Perce Tribal Hat. (fall) | 1.0 | 0.9886 | 0.9622 |
| RapCWST | Lolo Cr. (spring) | 1.0 | 0.9945 | 0.9708 |
| RapCWST | Newsome Cr. (spring) | 1.0 | 0.9982 | 0.9773 |
| RapCWST | Dworshak Hat. (spring) | 1.0 | 0.9940 | 0.9775 |
| RapCWST | Red River (spring) | 1.0 | 0.9949 | 0.9834 |
| RapCWST | Powell Trap (spring) | 1.0 | 0.9946 | 0.9541 |
| RapCWST | S. Fork Clearwater R. (spring) | 1.0 | 0.9961 | 0.9576 |
| RapCWST | Rapid River Hat. (spring) | 1.0 | 0.9990 | 0.9871 |
| MFSalST | Big Creek a (spring) | 1.0 | 0.9983 | 0.9973 |
| MFSalST | Big Creek b (spring) | 1.0 | 0.9988 | 0.9977 |
| SFSalST | Johnson Cr. (spring) | 1.0 | 0.9977 | 0.9888 |

| SFSalST | Secesh R. (spring) | 1.0 | 0.9952 | 0.9937 |
|---|---|---|---|---|
| SFSalST | McCall Hat. (spring) | 1.0 | 0.9973 | 0.9882 |
| upSalST | Sawtooth Hat. (spring) | 1.0 | 0.9920 | 0.9879 |
| upSalST | W. Fork Yankee Fork (spring) | 1.0 | 0.9992 | 0.9989 |
| upSalST | E. Fork Yankee Fork (spring) | 1.0 | 0.9961 | 0.9922 |
| upSalST | Pahsimeroi Hat. (spring) | 1.0 | 0.9976 | 0.9967 |
| MFSalST | Marsh Cr. (spring) | 1.0 | 0.9966 | 0.9963 |
| CalOT | Battle Cr. (spring) | 1.0 | 0.9996 | 0.9995 |
| CalOT | Butte Cr. (spring) | 1.0 | 1.0000 | 0.9999 |
| AKST | Klukshu R. | 1.0 | 1.0000 | 0.9999 |
| AKST | Situk R. | 1.0 | 1.0000 | 0.9999 |

Estimates of stock composition for the summer run mixture (n = 450) indicated the majority (90.2%) was comprised of mid/upper Columbia River (URB stock) as shown in Figure 1a. Small contributions were attributed to ocean-type stocks in the Klickitat River (1.7%) and the Snake River (1.6%), as well as some late run stream-type stocks from the mid/upper Columbia River (1.4%) and Salmon River (4.5%). Stock composition of the fall run mixture (n = 515) changed considerably from the summer run, but was still dominated by mid/upper Columbia River ocean-type (70.1%; Figure 1b). Snake River fall Chinook salmon (ESA listed stock) comprised a much larger proportion of the fall mixture (21.7%), and slight contributions from the Deschutes River (3.0%), Klickitat River (3.7%), and lower Columbia Tules (1.1%).

The likelihood of each individual belonging to either spring, summer, or fall run was determined from each multi-locus genotype in the software program STRUCTURE (Pritchard et al. 2000). In Figure 2, assignment of all individuals collected in 2005 throughout the entire run (early April to late October) show a transition to summer run in early June and the composition from June 1-15 was predominantly summer/fall run stocks.

**Figure 1.** Stock composition estimates of summer (a), and fall (b) runs of Chinook salmon collected at Bonneville Dam in 2005.

## a.) Summer run (6/16/05 to 7/19/05)



n = 450
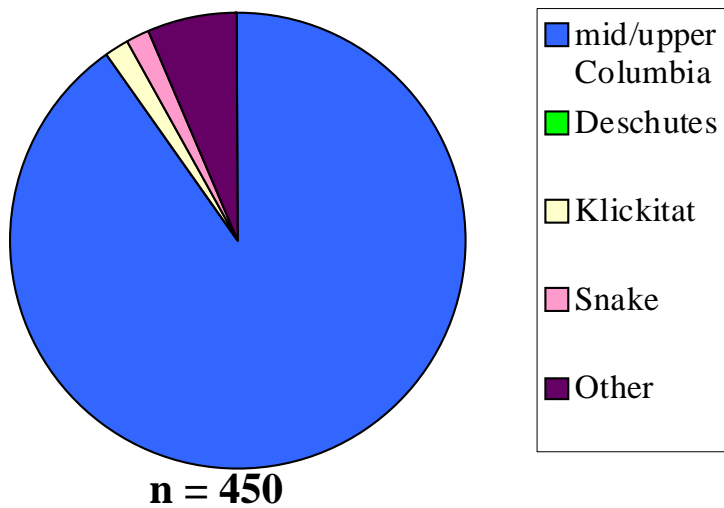
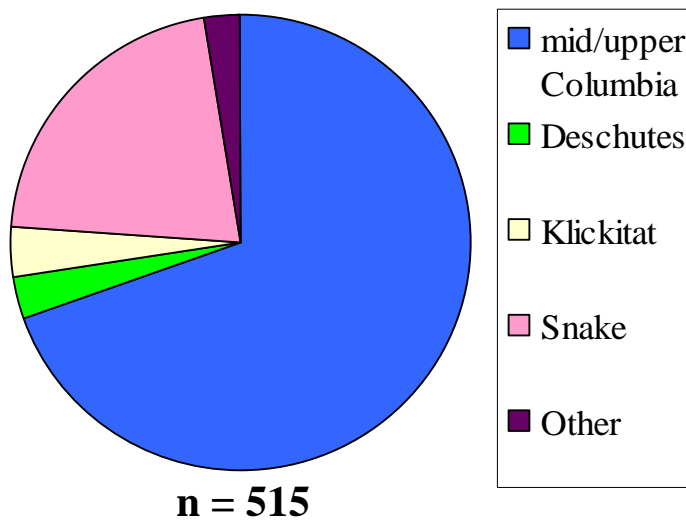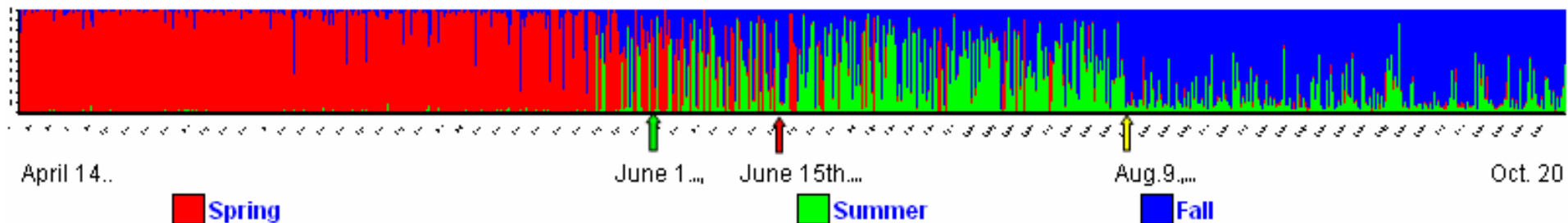## b.) Fall run (9/9/05 to 10/20/05)



n = 515

**Figure 2**. Likelihood of assignment of individual Chinook salmon to either, spring, summer, or fall run type, as collected throughout the entire run of 2005. Each vertical bar represents an individual fish and its assignment likelihood to one of the three runs based on genetic identity. Typical cut-off dates for run types are given below the figure as well as the revised cut-off date (June 15) for spring run in 2005.



April 14..                                                    June 1..,    June 15th....                                    Aug.9.,...                                                    Oct. 20

■ Spring                                              ■ Summer                                    ■ Fall

**Discussion**

The primary objective of this project was to genotype unknown Chinook salmon collected at Bonneville Dam and determine the composition of these mixed stock samples. We successfully completed this objective and closely followed the anticipated time schedule from the proposal. Estimates of composition of the summer and fall mixtures confirm that mid and upper Columbia River stocks dominate these runs. However, some ESA listed stocks are present and can be taken into account by managers for determining timing of commercial, sport, and tribal harvest. In particular, the Snake River fall stock was strongly represented in the mixture collected from September 9th to October 20th and may account for a significant portion of upriver fisheries. For this type of GSI information to be fully utilized by managers, much larger sample sizes and more narrow time windows need to be analyzed.

A secondary objective was to evaluate the cut-off for the start of the summer run over Bonneville Dam. The run timing of spring Chinook salmon has been delayed in recent years relative to the 10 year average peak return time. In 2005, estimates of stock composition show a large increase in the proportion of summer run Chinook when the cut-off date for spring Chinook was shifted to June 15th. In Figure 2, assignment of all individuals collected in 2005 throughout the entire run (early April to late October) confirm that the transition to summer run was more appropriately June 1 rather than the revised date of June 15. Thus, in 2005 the run of spring Chinook was over-estimated and summer Chinook were under-estimated.

*Quality Control*

Genetic data was tested under standard quality control procedures in CRITFC's

genetic laboratory. This includes confirmation of raw genotypes through repetitive

genotyping, positive and negative controls, and automated allele conversion. Further,

data was compared to initial baseline data generated in our laboratory to ensure

consistency of new baseline data. Mixture simulations were completed to determine the

power of the baseline for estimating composition of mixed stock samples. Simulations

indicated reasonably high power for GSI applications such as the current study.

*Project Benefits / Monitoring and Evaluation*

Estimates of stock composition and run timing with genetic stock ID provides a

tool to better manage fisheries and harvest opportunities. Genetic identification of each

run type and population will allow us to determine the stock composition of the different

runs through Bonneville Dam with greater accuracy than current methods. Utilization of

these advanced technologies offers tremendous improvement in the information that

managers need to appropriately define harvest slots and limits, and limiting the impact to

ESA listed stocks while targeting sustainable populations (Banks 2005). However, much

greater effort needs to be devoted towards sampling, genotyping, and GSI analysis for

further defining stock run timing over Bonneville Dam. While the current data provides

a general reference of stock run timing over Bonneville Dam, larger sample sizes will

allow a more thorough evaluation of narrow windows of time (i.e., weekly estimates of

stock composition). Current laboratory techniques also allow the possibility of "real-

time" GSI estimates that could be available to managers within 3-5 days after sampling. This suggests that if given adequate priority and funding, GSI information could be used for in-season fisheries management in the Columbia River Basin.

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